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Inhibition of morphine tolerance and dependence by MS-153, a glutamate transporter activator

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Abstract

We investigated the effects of (*R*)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153), which is reported to accelerate glutamate uptake, on the development of morphine tolerance and physical dependence in mice. For the induction of morphine tolerance and dependence, mice were twice daily treated with morphine (10–45 mg/kg, s.c.) for 5 days. First, co-administration of MS-153 (12.5 mg/kg, s.c.) did not affect the morphine's potency for its acute antinociceptive effect (1 and 3 mg/kg, s.c.). Next, co-administrations of MS-153 (1, 3 and 12.5 mg/kg, s.c.) during repeated morphine treatments significantly attenuated the development of tolerance to the antinociceptive effect of morphine (3 mg/kg, s.c.) and suppressed the naloxone (10 mg/kg, i.p.)-precipitated withdrawal signs (jumps and body weight loss). The inhibitory effect of MS-153 on the withdrawal signs was due to the attenuation of the development of dependence rather than that of expression of withdrawal signs. These results suggest that MS-153, a glutamate transporter activator, has an inhibitory effect on the development of morphine tolerance and physical dependence.

Keywords: MS-153; Morphine tolerance; Morphine dependence; Glutamate; Glutamate transporter

1. Introduction

Although morphine is widely used in the clinical management of pain, repeated use may lead to the development of tolerance and dependence, which limits its usefulness. Although the mechanisms of the development of tolerance to and dependence on morphine have been vigorously investigated, it still remains unclear. Recent evidence supports the involvement of glutamate, an excitatory neurotransmitter, in them (Zhu et al., 1998). It has been shown that several non-competitive and competitive NMDA receptor antagonists such as (+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cycloheptan-5,10-imine (MK-801) (Marek et al., 1991a,b; Trujillo and Akil, 1991), ketamine (Koyuncuoglu et al., 1990; Shimoyama et al., 1996) and (+)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid (LY274614) (Rasmussen et al., 1991), AMPA receptor antagonists (Rasmussen et al., 1996; McLemore et al., 1997), and metabotropic glutamate receptor antagonists (Fundytus and Coderre, 1994; Fundytus et al., 1997) attenuated the development of morphine tolerance and physical dependence without affecting the antinociceptive effect of morphine. Furthermore, morphine withdrawal-induced hyperactivity of locus coeruleus neurons was reported to be attenuated by intracoerulear injection of kynurenic acid and antagonists selective for either NMDA or non-NMDA glutamate receptors (Akaoka and Aston-Jones, 1991). Recently, neurochemical studies using in vivo microdialysis method has directly shown an elevation of extracellular glutamate level within the locus coeruleus during naloxone-precipitated morphine withdrawal (Aghajanian et al., 1994; Zhang et al., 1994). Indeed, ketamine is clinically used to potentiate morphine's analgesic effect and to attenuate morphine tolerance and dependence (Yang et al., 1996; Bell, 1999), although its clinical utility is limited by its psychotomimetic side effects.

(*R*)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153), a novel cerebroprotective agent, has been shown to reduce the area of cerebral infarction and to minimize neurological deficits induced by middle cerebral artery occlusion in rats (Umemura et al., 1996; Kawazura et al., 1997). It has been reported that MS-153 reduced the elevation of extra-

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cellular glutamate level at the ischemic penumbra zone during occlusion of the middle cerebral artery (Umemura et al., 1996). Recently, Shimada et al. (1999) found that MS-153 accelerated glutamate uptake through a glial glutamate transporter (GLT-1) expressed in COS-7 (CV-1 Origin, SV40) cells, and inhibited the depolarization- and ischemia-induced efflux of glutamate, but not other amino acids, in rat hippocampal slices. These findings suggest that MS-153 could be used as one of glutamate transporter activator.

In the present study, to examine the role of glutamate transporter in the development of tolerance to and physical dependence on morphine, we investigated the effects of co-administration of MS-153 with morphine on the development of morphine tolerance and physical dependence in mice.

2. Materials and methods

2.1. Animals

Male ddY mice weighing 20–24 g were used. They were kept at a constant ambient temperature of 23 ± 1°C under a 12-h light/dark cycle with free access to food and water.

2.2. Materials

MS-153 was a gift from the Life Science Laboratory of Mitsui Chemical (Chiba, Japan). Morphine hydrochloride and naloxone hydrochloride were purchased from Takeda Chemical Industries (Osaka, Japan) and Sigma (St. Louis, USA), respectively. All drugs were dissolved in 0.9% saline.

2.3. Induction of morphine tolerance and dependence

For the induction of tolerance to and dependence on morphine, the mice were subcutaneously injected with morphine hydrochloride twice daily at 9:00 and 19:00 h for 5 days, as described previously (Maldonado et al., 1989) with some modifications. The morphine hydrochloride dose was progressively increased as follows: day 1, 10 mg/kg (only one administration at 19:00 h); day 2, 15 and 20 mg/kg; day 3, 25 and 30 mg/kg; day 4, 35 and 40 mg/kg; day 5, 45 mg/kg (only one administration at 9:00 h), respectively.

2.4. Measurement of nociceptive threshold

The nociceptive threshold of the tail for mechanical stimulation was measured using a pressure analgesimeter (Ugo Basil, Milan, Italy) with a wedge-shaped pusher at a loading rate of 32 g/s, and the pressure eliciting tail withdrawal was determined as a nociceptive threshold. The mice were habituated to the procedure for measuring the threshold three times. Next day, the threshold of each animal was measured following three additional habituation procedures, and the threshold value was taken as a base value at time 0. Within 10 min after measuring the base value, the drugs were administered, and the nociceptive thresholds were measured at 15, 30, 60, 90, 120 and 150 min after the administration. The area under the curve (AUC) values were obtained by calculating the area between the base value and the curve for the time course of the nociceptive threshold from 0 to 150 min after the administration.

2.5. Measurement of naloxone-precipitated morphine withdrawal

Development of morphine dependence was assessed from naloxone-precipitated withdrawal jumps and body weight loss 2 h after the final administration of morphine on day 5, as described previously (Katsumata et al., 1995).

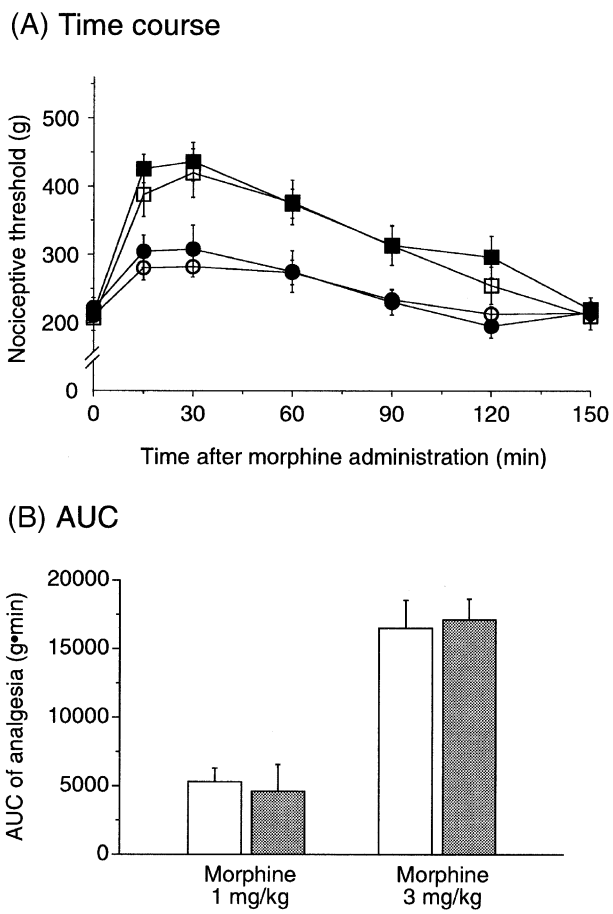

Fig. 1. Effects of MS-153 on the acute antinociceptive effect of morphine in the mouse tail pressure test. (A) Morphine at doses of 1 (●, ○) and 3 (■, □) mg/kg were administered subcutaneously at time 0 in combination with or without MS-153 at a dose of 12.5 mg/kg, respectively. The nociceptive threshold at each point is presented as the mean ± S.E.M of 9–11 animals. (B) The magnitudes of the effects of MS-153 on the acute morphine antinociception are presented as the AUC values of analgesia. Morphine at doses of 1 and 3 mg/kg were co-administered with (shaded bar) or without (open bar) MS-153 at a dose of 12.5 mg/kg, respectively.

Briefly, each mouse was placed into a plexiglass cylinder 25 cm in diameter and 30 cm in height for 30 min in order to acclimatize it to the experimental environment. After the 30-min habituation period, the mice were administered

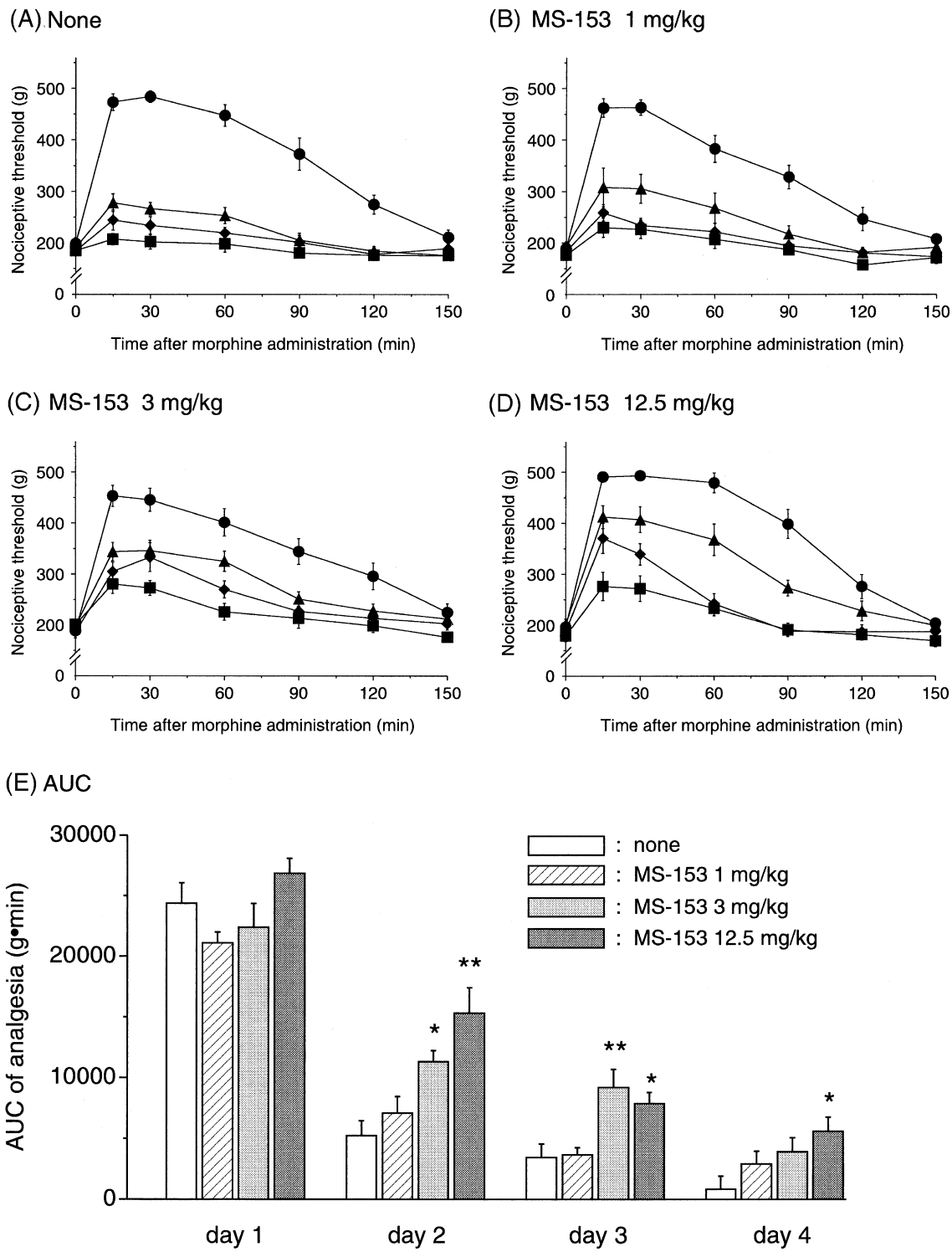

Fig. 2. Effects of co-administrations of MS-153 with morphine on the development of tolerance to the antinociceptive effects of morphine. Mice were repeatedly treated with morphine at progressively increasing doses in combination with MS-153 at doses of 1 (B), 3 (C) and 12.5 (D) mg/kg or without (A) as described in Materials and methods. For measurement of tolerance to the morphine antinociception, the antinociceptive effects of single s.c. administration of morphine at a dose of 3 mg/kg were measured in the mouse tail pressure test on day 1 (●), 2 (▲), 3 (♦) and 4 (■). The nociceptive threshold at each point is presented as the mean ± S.E.M of 9–11 animals. (E) The magnitudes of the effects of MS-153 on the development of morphine tolerance on day 1–4 are presented as the AUC values of analgesia. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group on each day (Dunnett's multiple comparison test).

with naloxone (10 mg/kg, i.p.). Immediately after naloxone was administered, the mice were returned to the plexiglass cylinder and the number of jumps was counted for 40 min. Body weight was measured immediately before, and 40 min after naloxone administration.

2.6. Treatment schedule

We carried out three experiments. In experiment 1, to investigate the effects of MS-153 on the morphine antinociception, mice not repeatedly treated with morphine were acutely administered a single injection of MS-153 (12.5 mg/kg, s.c.) in combination with morphine (1 and 3 mg/kg, s.c.). In experiment 2, to evaluate the effects of MS-153 on the development of morphine tolerance and the naloxone-precipitated withdrawal signs, mice were given MS-153 (1, 3 and 12.5 mg/kg, s.c.) in combination with repeated morphine treatments every time. In this paradigm, analgesic response to morphine (3 mg/kg, s.c.) was assessed on day 1, 2, 3 and 4 at 14:00 h. In experiment 3, to investigate whether MS-153 affects the development of dependence on morphine or the expression of naloxone-precipitated withdrawal signs, mice were injected MS-153 (10 mg/kg, s.c.) either for day 1–5 (i.e. every time), only for the first 4 days (i.e. excluding on day 5) or only on day 5 (2 h before the naloxone challenge) in combination with repeated morphine treatments.

2.7. Statistical analysis

All results are expressed as means ± S.E.M. The statistical significance was calculated using a one-way analysis of variance (ANOVA) test followed by Dunnett's multiple comparison test or Mann–Whitney *U*-test. Differences with $P < 0.05$ were considered significant.

3. Results

3.1. Effects of MS-153 on morphine antinociception

The effects of co-administration of MS-153 on the acute antinociceptive effect of morphine were investigated in naive mice not repeatedly treated with morphine. A single s.c. administration of morphine at doses of 1 and 3 mg/kg elevated the nociceptive threshold, which peaked at 15–30 min after the administration. Co-administration of MS-153 at a dose of 12.5 mg/kg did not alter the acute antinociceptive effects of morphine at both doses of 1 and 3 mg/kg (Fig. 1).

3.2. Effects of co-administrations of MS-153 with morphine on the development of tolerance to morphine antinociception, and the naloxone-precipitated withdrawal signs

The effects of co-administrations of MS-153 with morphine on the development of tolerance to the antinociceptive effect of morphine were investigated on day 1–4. On day 1, a single s.c. administration of morphine at a dose of 3 mg/kg elevated the nociceptive thresholds in mice, which were not treated with repeated morphine yet. On day 2 and 3, repeated treatments with morphine rapidly reduced the antinociceptive effect, and on day 4, the antinociceptive effect completely disappeared as a result of the development of tolerance to morphine (Fig. 2A). Repeated co-administrations of MS-153 at doses of 1, 3 and 12.5 mg/kg with morphine reduced the development of tolerance to morphine (Fig. 2B–D). The AUC values of analgesic response of the groups co-administered with MS-153 at doses of 3 and 12.5 mg/kg on day 2 ($P < 0.05$ and $P < 0.01$, respectively) and day 3 ($P < 0.01$ and $P < 0.05$, respectively) and at a dose of 12.5 mg/kg on day 4 ($P < 0.05$) were significantly larger than that of the control group on each day (Fig. 2E).

On day 5, the effects of co-administrations of MS-153 with morphine on the naloxone-precipitated withdrawal

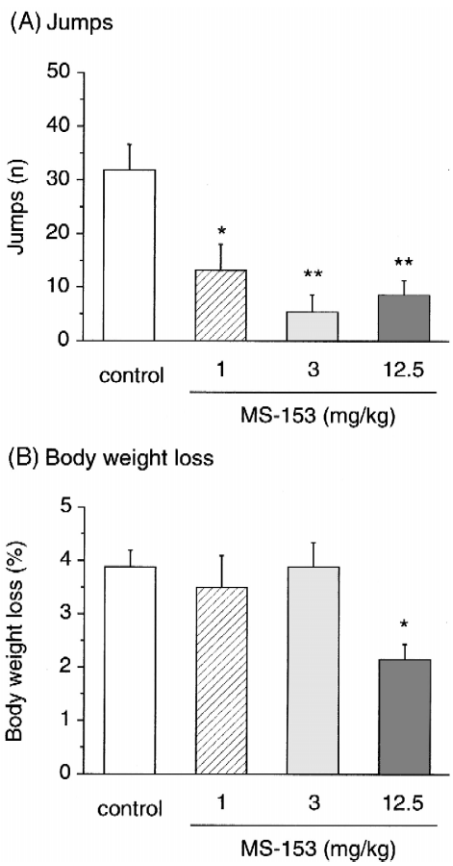

Fig. 3. Effects of co-administrations of MS-153 with morphine on the naloxone-precipitated withdrawal signs in morphine-dependent mice. Mice were repeatedly treated with morphine in combination with MS-153 at doses of 1, 3 and 12.5 mg/kg or without (control) as described in Materials and methods. On day 5, mice were administered with naloxone (10 mg/kg, i.p.) 2 h after the final administration of morphine, and the naloxone-precipitated withdrawal jumps (A) and body weight loss (B) were measured. Each column represents the mean ± S.E.M. of 9–11 animals during 40 min. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group (Dunnett's multiple comparison test).

signs were investigated. In the control group repeatedly treated with morphine alone, i.p. administration of naloxone (10 mg/kg) elicited withdrawal signs such as jumps and body weight loss. Repeated co-administrations of MS-153 at doses of 1, 3 and 12.5 mg/kg with morphine significantly suppressed the naloxone-precipitated jumps (1 mg/kg, $P < 0.05$; 3 and 12.5 mg/kg, $P < 0.01$) and body weight loss (12.5 mg/kg, $P < 0.05$) compared with those of the control group (Fig. 3).

3.3. Effects of co-administrations of MS-153 with morphine on the development of morphine dependence and the expression of naloxone-precipitated withdrawal signs

We examined whether repeated co-administrations of MS-153 with morphine affect the development of dependence on morphine or the expression of naloxone-precipitated withdrawal signs. Repeated co-administrations of MS-153 at a dose of 10 mg/kg with morphine for day 1–5 (every time) and only for day 1–4 (first 4 days) significantly suppressed the naloxone-precipitated withdrawal jumps ($P < 0.05$ and $P < 0.01$, respectively) and body weight loss ($P < 0.05$) compared with those of the control group. On the other hand, co-administration of MS-153 only on the day 5 (2 h before the naloxone challenge) had no significant effects on the naloxone-precipitated jumps and body weight loss, although it tended to suppress them (Fig. 4).

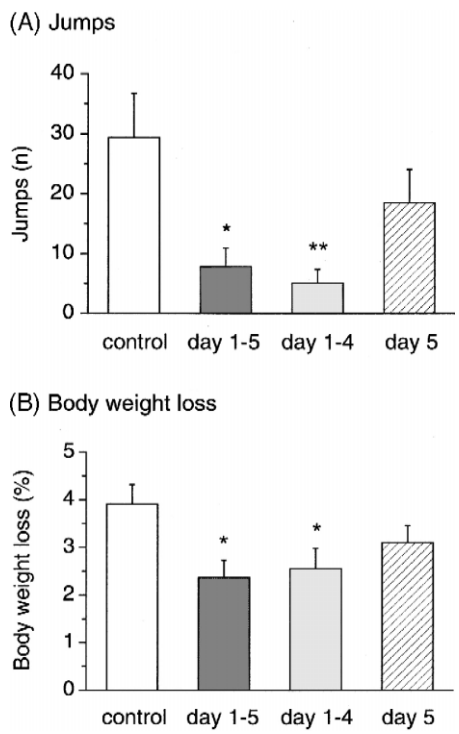

Fig. 4. Effects of MS-153 on the development of morphine dependence and the expression of morphine withdrawal. Mice were repeatedly treated with morphine in combination with MS-153 at a dose of 10 mg/kg either for day 1–5, for day 1–4, only on day 5, or without (control) as described in Materials and methods. On day 5, naloxone (10 mg/kg, i.p.)-precipitated withdrawal jumps (A) and body weight loss (B) were measured. Each column represents the mean ± S.E.M. of 10 animals during 40 min. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control group (Mann–Whitney *U*-test).

4. Discussion

In the present study, we have shown that co-administrations of MS-153 with morphine significantly attenuated the development of tolerance to the antinociceptive effect of morphine in the mouse tail pressure test. The inhibitory effect of MS-153 was observed at least for 4 days from starting the repeated morphine treatments. Furthermore, we found that MS-153 also significantly suppressed the naloxone-precipitated withdrawal signs (jumps and body weight loss) on day 5. When MS-153 was co-administered with morphine for day 1–5 and day 1–4, but not only on day 5, the naloxone-precipitated withdrawal signs were significantly suppressed. These data suggest that MS-153 attenuated the development of physical dependence on morphine, rather than the expression of naloxone-precipitated withdrawal signs in this time schedule. In addition, these inhibitory effects of MS-153 on the development of tolerance and physical dependence were not due to the reduction of the morphine's potency for its antinociceptive effect during repeated treatments by co-administration of MS-153, because MS-153 had no effects on the acute morphine antinociception, although we cannot rule out the possibility that repeated administration of MS-153 might affect the antinociceptive effects of morphine.

A growing body of evidence suggests that the excitatory amino acid, particularly glutamatergic, systems are involved in the development of morphine tolerance and dependence. The findings that the non-competitive NMDA receptor antagonists, MK-801, attenuated the development of morphine tolerance and physical dependence without affecting the antinociceptive effect of morphine (Marek et al., 1991a,b; Trujillo and Akil, 1991) have focused the interest of investigators in the involvement of glutamate. Similarly, several other non-competitive and competitive NMDA receptor antagonists (Koyuncuoglu et al., 1990; Shimoyama et al., 1996; Rasmussen et al., 1991), AMPA receptor antagonists (Rasmussen et al., 1996; McLemore et al., 1997) and metabotropic glutamate receptor antagonists (Fundytus and Coderre, 1994; Fundytus et al., 1997) have been reported to attenuated them. Furthermore, pretreatment with an antisense oligonucleotide to the NMDA-R1 (NR1) subunit attenuated naloxone-precipitated withdrawal signs (Zhu and Ho, 1998), and the expression of the NR1 subunit mRNA was increased in the locus coeruleus and the hypothalamic paraventricular nucleus of morphine-dependent rats (Zhu et al., 1999). These findings suggest that glutamatergic systems mediate the neural and behavioral plasticity responsible for morphine tolerance and dependence. MS-153, a novel cerebroprotective agent, has been reported to reduce the depolarization- and ischemia-in-

duced elevation of extracellular glutamate level by accelerating glutamate uptake (Umemura et al., 1996; Shimada et al., 1999), suggesting that MS-153 could modulate the glutamatergic system. Taken together, the inhibitory effects of MS-153 on the development of morphine tolerance and physical dependence are suggested to be due to the modulating effects of MS-153 on the extracellular glutamate level during repeated morphine treatment.

It is well known that extracellular glutamate by release from nerve terminals is counterbalanced by glutamate transporters in neurons and glial cells, thereby terminating the glutamatergic signal transmission and protecting neurons from an excitotoxic action of glutamate (Kanner and Schuldiner, 1987; Kanai et al., 1993). To date, five subtypes of high-affinity glutamate transporters have been cloned and characterized (Seal and Amara, 1999), namely glutamate transporter (GLT-1) (Pines et al., 1992), glutamate/aspartate transporter (GLAST) (Storck et al., 1992), excitatory amino acid carrier 1 (EAAC1) (Kanai and Hediger, 1992), excitatory amino acid transporter 4 (EAAT4) (Fairman et al., 1995) and excitatory amino acid transporter 5 (EAAT5) (Arriza et al., 1997). GLT-1 and GLAST are mainly expressed in astrocytes, whereas EAAC1 and EAAT4 are mainly in neurons (Rothstein et al., 1994; Lehre et al., 1995; Furuta et al., 1997; Schmitt et al., 1997). It has been shown that the glial glutamate transporters, GLT-1 and GLAST, play important roles in maintaining the extracellular glutamate concentration below the neurotoxic level, rather than neuronal glutamate transporters (Rothstein et al., 1996; Gegelashvili and Schousboe, 1997; Tanaka et al., 1997). Recently, MS-153 is reported to accelerate the L-[^{3}H]glutamate uptake in COS-7 cells expressing GLT-1 by enhancing the activity of GLT-1, but not [^{3}H]gamma-aminobutyric acid (GABA) uptake in COS-7 cells expressing glial GABA transporter (Shimada et al., 1999). Furthermore, MS-153 has no effects on NMDA or AMPA glutamate receptors (Akaike et al., 1993) and Ca^{2+} channels (Shimada et al., 1999), although the effects of MS-153 on the other glutamate transporters are not yet determined. Taken together, the present results suggest that the glial glutamate transporter GLT-1 is involved in the development of morphine tolerance and dependence. Furthermore, our findings that the expression of GLT-1, but not GLAST, mRNA was decreased in several brain regions of morphine-dependent rats, compared with naive rats (unpublished data) support this possibility.

In conclusion, we have shown that co-administration of MS-153, which is a glutamate transporter activator, with morphine reduced the development of tolerance to and physical dependence on morphine, suggesting that the glutamate transporter GLT-1 might play a modulating role in them. Our results suggest the possibility that glutamate transporter activators such as MS-153 can be used as co-medications with morphine in the treatment of chronic pain.

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